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64. (Amended) The method of Claim 11, wherein the peripheral blood is drawn in an anticoagulant substance and, prior to covering the cell separation medium, diluted with a diluent at a ratio of about 1:1.

69. (Amended) The method of Claim 1, wherein the tumor cells are separated from telomerase-positive non tumor cells.

70. (Amended) The method of Claim 1, further comprising, before step (i), adjusting the density of the cell separation medium and thereby separating tumor cells from telomerase-positive non tumor cells.

REMARKS

A check for the fee for a three month extension of time accompanies this response. Any fees that may be due in connection with filing this paper or with this application may be charged to Deposit Account No. 50-1213. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

Claims 1-19, 21-24, 26-38, 52-67 70-86 are pending. Claims 20, 25, 39-51 and 68, are cancelled without prejudice or disclaimer. Claims 39-51 and 68 are drawn to non-elected subject matter. Claim 25, which is duplicative of Claim 11, is cancelled. Applicant reserves the right to file divisional applications to the non-elected and cancelled subject matter.

Claims 1, 21-24, 26-30, 34, 61, 62, 64, 69 and 70 are amended. Claim 1 is amended to incorporate steps of cancelled claim 20. The remaining claim amendments correct obvious errors and inconsistencies, such as claim dependency, and to provide proper antecedent basis.

Claims 71-86, which find basis in the application as originally filed, are added. For example, particular basis for claim 71 and claims dependent thereon can be found in the claims as originally filed and in the specification, *e.g.*, at page 15, lines 24-34; page 17, lines 22-38 and page 24, lines 20-27 of the specification. Particular basis for claim 72 and claims dependent thereon can be found, for example, at page 5, lines 24-33; page 17, line 22 to page 18, line 14 and page 18, line 24 to page 19, line 21 of the specification. No amendments have been made to obviate prior art and no new matter is added.

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Included as an attachment is a marked-up version of the claims that are amended, per 37 CFR §1.121.

**THE REJECTION OF CLAIMS 1, 2, 4, 7-11, 14-17, 37, 38, 52-56 and 67
UNDER 35 U.S.C. §102(e)**

Claims 1, 2, 4, 7-11, 14-17, 37, 38, 52-56 and 67 are rejected under 35 U.S.C. 102(e) as being anticipated by Cech *et al.* (U.S. Patent No. 6,166,178; filed November 19, 1997) because that Cech *et al.* allegedly discloses methods of quantitating tumor cells in a body fluid by concentrating tumor cells in a sample of body fluid, amplifying mRNA coding for the catalytic subunit of telomerase (hTERT) and quantitatively determining the amount of amplified nucleic acid. Specifically, it is urged that Cech *et al.* discloses (1) methods of diagnosing cancer in a patient by detecting a hTERT gene product in a biological sample (exemplified at col. 104, lines 65-68) obtained from the patient (col. 6, lines 20-40); (2) that detection of the hTERT gene, mRNA or protein level above a standard range is indicative of the presence of telomerase-positive cells such as tumor cells (col. 99, lines 5-20); (3) that cells or tissues may be fractionated, *e.g.*, by a cell sorter, prior to analysis (col. 105, lines 10-12; (4) primers useful for PCR amplification of hTERT (col. 107, lines 1-5); (5) analysis of the PCR amplified products (col. 108, lines 1-5); and (6) co-amplification reactions for normalization of the amount of hTERT in the sample to the cell number in the sample (col. 108, lines 45-65). Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Relevant Law

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. In re Spada, 15 USPQ2d 1655 (Fed. Cir, 1990), In re Bond, 15 USPQ 1566 (Fed. Cir. 1990), Soundsciber Corp. v. U.S., 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, Richardson v. Suzuki Motor Co., 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll

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limitations in the claims must be found in the reference, since the claims measure the invention". In re Lang, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). Moreover it is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference.

Lindemann Maschinen-fabrik GmbH v. American Hoist and Derrick Co., 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984). Further, the reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. In re Oelrich, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

The Claims

All the rejected claims (Claim 1 and dependents 2, 4, 7-11, 14-17, 37, 38, 52-56 and 67) are directed to a method for the quantification of tumor cells in a body fluid by (a) concentrating tumor cells in a sample of a body fluid by covering a cell separation medium with a layer of the body fluid, centrifuging the cell separation medium covered with the body fluid and collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid; (b) specifically amplifying, from the concentrated tumor cells, mRNA coding for the catalytic subunit of telomerase; and (c) quantitatively determining the amount of amplified nucleic acid, thereby quantifying tumor cells in a body fluid. Claim 2 further specifies that prior to amplification, cDNA is prepared from the mRNA. Claim 4 specifies that the sample of Claim 1 is purified, and Claims 52 and 53 further specify particulars of purification. Claims 7-11 and 54-56 specify particulars for the quantitative determination of telomerase-encoding nucleic acid. Claims 14 and 15 specify particulars for amplification of the mRNA. Claim 16 is directed to the method of Claim 1 in which the sample is blood that is depleted in stem cells and/or activated immune cells, and Claim 17 specifies that the sample is blood from which tumor cells are concentrated. Claims 37 and 38 specify types of tumor cells. Claim 67 is directed to the

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method of Claim 1 where the tumor cells are derived from micrometastases of malignant tumors.

Added Claim 71 and claims dependent thereon are directed to a method for the quantification of tumor cells in a body fluid, by (a) concentrating and separating the tumor cells from telomerase-positive non-tumor cells in a sample of a body fluid; (b) specifically amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase; and (c) quantitatively determining the amount of amplified nucleic acid, thereby quantifying tumor cells in a body fluid. Added Claim 72 and claims dependent thereon are directed to a method for the quantification of tumor cells in a body fluid that includes the steps of (a) concentrating tumor cells in a sample of a body fluid by treating the sample of body fluid with a cell separation medium; (b) specifically amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase; and (c) quantitatively determining the amount of amplified nucleic acid, thereby quantifying tumor cells in a body fluid.

Thus, all the claims include as one element, a method for the quantification of tumor cells in a body fluid. Further, all of the claims recite the steps of (i) concentrating the tumor cells by covering a cell separation medium with a layer of the body fluid, centrifuging the cell separation medium covered with the body fluid and collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid; (ii) specifically amplifying, from the concentrated tumor cells, the mRNA for the catalytic subunit of telomerase; (iii) quantifying the amplified mRNA for the catalytic subunit of telomerase and correlating the quantity of amplified mRNA with the quantity of tumor cells in a body fluid. Added Claim 71 specifies that in the first step in a method for quantification of tumor cells in a body fluid, the tumor cells are concentrated and separated from telomerase positive non-tumor cells. Added Claim 72 specifies that in a method for quantification of tumor cells in a body fluid, the tumor cells are concentrated in the first step by treating the sample of body fluid with a cell separation medium.

Differences between the claims and the disclosure of Cech *et al.*

Cech *et al.*

Cech *et al.* discloses nucleic acids encoding the catalytic subunit of telomerase and related polypeptides; methods for determining modulators of the activity of the catalytic subunit of telomerase; and diagnosing telomerase-related conditions such as cancer by obtaining biological samples and detecting cells containing a "high level" of the catalytic subunit of telomerase. Cech *et al.* discloses that cancer can be diagnosed in a patient by obtaining a biological sample containing at least one cell; determining the amount of catalytic subunit of telomerase gene product in the cell, and comparing the amount of gene product in the cell with a normal value for the cell, where an amount that is elevated relative to the normal value is indicative of cancer (col. 6, lines 42-48). Cech *et al.* further discloses that co-amplification with a control polynucleotide of known concentration or copy number can be used to quantitate the amount of catalytic subunit of telomerase mRNA, or, alternatively, co-amplification of the control can be used for normalization to the cell number in the sample (col. 108, lines 45-65). The "biological samples" listed in Cech *et al.* include cells (whole cells, cell fractions, cell extracts, and cultured cells or cell lines), tissues (including biopsy samples), body fluids and cells collected therefrom (col. 104, line 59 to col. 105, line 1). Cech *et al.* discloses that in some cases, the cells or tissues may be fractionated before analysis using, *e.g.*, a fluorescence-activated cell sorter to sort cells according to characteristics such as expression of a surface antigen (col. 105, lines 10-15).

Cech *et al.* does not disclose a method of quantification of tumor cells in a body fluid in which the tumor cells in the body fluid are concentrated. Cech *et al.* discloses quantitation of the amount of amplified catalytic subunit of telomerase mRNA in a biological sample and comparing the amount of gene product in the sample with a normal value for the sample, where an amount that is elevated relative to the normal value is indicative of cancer. Cech *et al.* also discloses normalization of amount of coamplified control DNA to the total

number of cells in a sample rather than to the amount of amplified catalytic subunit of telomerase mRNA. Cech *et al.*, however, **does not** disclose absolute quantitation of the catalytic subunit of telomerase mRNA that is specifically amplified from concentrated tumor cells of a body fluid as an indication of the quantity of tumor cells in a body fluid. Cech *et al.* also does not disclose correlating the amount of the specifically amplified catalytic subunit of telomerase mRNA to the number of tumor cells in the sample. Further, with respect to claim 1 and claims dependent thereon, there is no disclosure in Cech *et al.* of concentrating tumor cells that are present in a body fluid by treatment of the body fluid with a cell separation medium (*i.e.*, a suitable liquid of desired density as defined in the specification, *e.g.*, at page 18, lines 24-31) so that the amplification product of the catalytic subunit of telomerase mRNA is specifically obtained from these concentrated tumor cells and is used to quantitate the tumor cells. Cech *et al.* certainly does not disclose further centrifugation steps to concentrate tumor cells by treatment with a cell separation medium such as the steps of covering the cell separation medium with a layer of the body fluid, centrifuging the cell separation medium covered with the body fluid and collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid. With respect to added Claim 71, Cech *et al.* does not disclose separation of tumor cells in a body fluid from telmerase positive non-tumor cells.

Therefore, Cech *et al.* does not anticipate any of the rejected claims and claim 72 and claims dependent thereon, which all require as elements: (1) the quantification of tumor cells in a body fluid by: (2) concentrating the tumor cells by treatment with a cell separation medium or by covering a cell separation medium with a layer of the body fluid, centrifuging the cell separation medium covered with the body fluid and collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid and (3) specifically amplifying, from the concentrated tumor cells, mRNA coding for the catalytic

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subunit of telomerase; so that the quantity of amplified catalytic subunit of telomerase can be correlated to the number of tumor cells in the body fluid.

With respect to added Claim 71 and claims dependent thereon, Cech *et al.* does not disclose a method for the quantification of tumor cells in a body fluid in which the tumor cells are concentrated and separated from the telomerase positive non-tumor cells.

Therefore, since anticipation requires disclosure in a single reference of all elements as claimed, and Cech *et al.* does not disclose all elements as claimed in any pending claims, Cech *et al.* does not anticipate any of the pending claims.

THE REJECTION OF CLAIMS 3, 5, 6, 12, 13, 18-36, 57-66, 69 and 70 UNDER 35 U.S.C. § 103(a)

A. REJECTION OF CLAIMS 18-28, 34-36, 60-64, 69 and 70 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF VAN VLASSELAER

Claims 18-28, 34-36, 60-64, 69 and 70 are rejected under 35 U.S.C. §103(a) over Cech *et al.* in view of Van Vlasselaer (U.S. Patent No. 5,648,223) because Cech *et al.* allegedly teaches a method of quantitating tumor cells in a body fluid, specifically, (1) methods of diagnosing cancer in a patient by detecting a catalytic subunit of telomerase (hTERT) gene product in a biological sample (exemplified at col. 104, lines 65-68) obtained from the patient (col. 6, lines 20-40); (2) that detection of the hTERT gene, mRNA or protein level above a standard range is indicative of the presence of telomerase-positive cells such as tumor cells (col. 99, lines 5-20); (3) that cells or tissues may be fractionated, *e.g.*, by a cell sorter, prior to analysis (col. 105, lines 10-12; (4) primers useful for PCR amplification of hTERT (col. 107, lines 1-5); (5) analysis of the PCR amplified products (col. 108, lines 1-5); and (6) co-amplification reactions for normalization of the amount of hTERT in the sample to the cell number in the sample (col. 108, lines 45-65); and Van Vlasselaer allegedly teaches methods for enriching tumor cells by adjusting the density of cell separation media that

are "routinely used in the art", such as Percoll and Ficoll, according to the density of the cell type. The Examiner alleges that it would have been *prima facie* obvious to one of ordinary skill in the art to have modified the method of quantitation of tumor cells allegedly taught by Cech *et al.* with the tumor cell enrichment method using a cell separation medium allegedly taught by Van Vlasselaer, to arrive at the instantly claimed subject matter. Specifically, it is alleged that Van Vlasselaer teaches (1) Percoll and Ficoll (recited in instant claim 23) as cell separation media that are "routinely used in the art;" (2) providing a substance that prevents platelets from sticking to the tumor cells (recited in claim 24) and to remove the platelets as "routinely practised in the art" (3) that the density of the cell separation medium is adjusted to the density of the cell type, where the cell density is determined by "routine experimentation" (limitations of instant claims 21, 22, 24 and 62-64); and (4) that peripheral blood can be collected in anti-coagulant containing tubes (recited in claims 24-27). Reconsideration and withdrawal of this rejection is respectfully requested in view of the amendments herein and the following remarks. It is respectfully submitted that this rejection has been rendered moot with respect to Claims 20 and 25, which have been cancelled.

Relevant law

In order to set forth a *prima facie* case of obviousness under 35 U.S.C. § 103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (*ACS Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must actually teach or suggest the claimed subject matter. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. *Ex parte Gerlach*, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art" *In re Keller*, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by

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combining the teachings of the prior art to produce the claimed subject matter, absent some teaching or suggestion supporting the combination (*ACS Hosp. Systems, Inc. v Montefiore Hosp.* 732 F.2d 1572, 1577. 221 USPQ 929, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" *W.L. Gore & Associates, Inc. v Garlock Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

The prior art must provide a motivation whereby one of ordinary skill in the art would have been led to do that which the applicant has done. *Stratoflex Inc. v Aeroquip Corp.*, 713 F.2d 1530, 1535, 218 USPQ 871, 876 (Fed. Cir. 1983). In addition, the mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 USPQ 1783 (Fed. Cir. 1992).

Also, it is impermissible to ignore the advantages, properties, utilities and unexpected results that flow from the claimed invention; they are part of the invention as a whole. *In re Sernaker*, 702 F.2d 989, 217 USPQ 1 (Fed. Cir. 1983). Unexpected properties must always be considered when determining obviousness. A compound's structure and properties are inseparable so that unexpected properties are part of the subject matter as a whole. *In re Papesh*, 315 F.2d 381, 137 USPQ 43 (CCPA 1963).

Analysis

The Claims

Claim 18 is directed to the method of Claim 1 where the cells in the sample are cultivated under conditions that are unfavorable for telomerase-positive nontumor cells and the tumor cells survive. Claim 19 specifies that the duration of the cultivation in the method of Claim 18 is such that nontumor cells die and tumor cells survive. Claim 60 specifies that stem cell and/or activated

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immune cell depletion from the body fluid blood may be effected by immunoabsorption, and Claim 61 as amended specifies that in added Claim 71, the tumor cells are concentrated by immunoabsorption.

Dependent Claims 21, 62 and 63 specify the density of the cell separation medium of Claim 1. Dependent Claim 22 specifies the time and speed of centrifugation, and dependent Claim 23 specifies that the cell separation medium used is Percoll or Ficoll. Claims 26 and 27 specify that the body fluid is peripheral blood and further specify that the peripheral blood can be either arterial or venous blood and may be drawn in an anticoagulant substance¹ and diluted with a diluent prior to covering the cell separation medium. Claim 64 is directed to the method of Claim 11 where the peripheral blood is drawn with an anticoagulant substance and, prior to covering the cell separation medium, diluted with a diluent at a ratio of about 1:1. Claim 24 specifies that the body fluid is blood and that prior to applying the sample to the cell separation medium, it is treated to prevent aggregation of platelets to tumor cells. Claim 28 specifies the types of body fluids that may be used in the method of Claim 1, and Claim 29 specifies that in the method of Claim 1, the centrifugation vessel is removed and cooled to prevent mixing of the cells. Claim 34 is directed to the method of Claim 1 in which a dye is added to the cell separation medium so as to distinguish the layer of cell separation medium from the layer of body fluid. Claim 35 is directed to the method of Claim 1 where the sample is blood and the method is performed on both arterial and venous blood samples and the results are compared to one another, and Claim 36 further specifies that blood sample is obtained from a finger pad. Claims 69 and 70 specify that in the method of Claim 1, the tumor cells are separated from telomerase-positive non tumor cells.

Added Claim 71 and claims dependent thereon are directed to a method for the quantification of tumor cells in a body fluid, by (a) concentrating and separating the tumor cells from telomerase-positive non-tumor cells in a sample of a body fluid; (b) specifically amplifying, from the tumor cells, mRNA coding

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for the catalytic subunit of telomerase; and (c) quantitatively determining the amount of amplified nucleic acid, thereby quantifying tumor cells in a body fluid. Added Claim 72 and claims dependent thereon are directed to a method for the quantification of tumor cells in a body fluid that includes the steps of (a) concentrating tumor cells in a sample of a body fluid by treating the sample of body fluid with a cell separation medium; (b) specifically amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase; and (c) quantitatively determining the amount of amplified nucleic acid, thereby quantifying tumor cells in a body fluid.

Thus, all the claims include as one element, a method for the quantification of tumor cells in a body fluid. Further, all of the claims include a steps of (i) concentrating the tumor cells regardless of tumor cell type by treatment with a cell separation medium; (ii) specifically amplifying, from the tumor cells, the mRNA for the catalytic subunit of telomerase; (iii) quantifying the amplified mRNA for the catalytic subunit of telomerase and correlating the quantity of amplified mRNA with the quantity of tumor cells in a body fluid. The dependent claims specify the particulars of concentration of the tumor cells by treatment with a cell separation medium; the centrifugation steps for concentration of tumor cells from a body fluid by applying a cell separation medium to the body fluid; that the body fluid is blood; or that the cells of the body fluid sample may be cultivated such that there is a selection against telomerase-positive nontumor cells and in favor of telomerase positive tumor cells. Claims 69 and 70 specify that the tumor cells are separated from the telomerase-positive non tumor cells. Added Claim 71 specifies that in the first step in a method for quantification of tumor cells in a body fluid, the tumor cells are concentrated and separated from telomerase positive non-tumor cells. Added Claim 72 specifies that in a method for quantification of tumor cells in a body fluid, the tumor cells are concentrated in the first step by treating the sample of body fluid with a cell separation medium.

Neither of the cited references teaches or suggests a method for the quantification of tumor cells in a body fluid. Therefore, a combination of the references cannot cure this deficiency. Further, neither of the cited references, singly or in combination, leads to the combination of steps for the quantification of tumor cells in a body fluid by: (1) concentrating tumor cells in the body fluid by treatment with a cell separation medium and (2) specifically amplifying, from the concentrated tumor cells, mRNA coding for the catalytic subunit of telomerase; so that the quantity of amplified catalytic subunit of telomerase can be correlated to the number of tumor cells in the body fluid. Furthermore, neither of the cited references, singly or in combination, teaches or suggests any cultivation or separation steps for selection of tumor cells over telomerase-positive non tumor cells.

Teachings of the cited art and differences from the claimed methods

Cech et al.

Cech et al. teaches nucleic acids encoding the catalytic subunit of telomerase and related polypeptides; methods for determining modulators of the activity of the catalytic subunit of telomerase; and diagnosing telomerase-related conditions such as cancer by obtaining biological samples and detecting cells containing a "high level" of the catalytic subunit of telomerase. *Cech et al.* teaches that cancer can be diagnosed in a patient by measuring the amount of catalytic subunit of telomerase in a patient's cell relative to the normal value for that cell, where an amount that is elevated relative to the normal value is indicative of cancer. *Cech et al.* further teaches that co-amplification with a control polynucleotide of known concentration or copy number can be used to quantitate the amount of catalytic subunit of telomerase mRNA.

Cech et al. does not teach or suggest a method of quantification of tumor cells in a body fluid in which the tumor cells are concentrated in the body fluid. *Cech et al.* teaches that quantitation of the amount of amplified catalytic subunit of telomerase mRNA in a biological sample may be indicative of cancer, if the

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amount of catalytic subunit of telomerase mRNA in the sample is elevated relative to normal values for that sample.

Cech *et al.* does not teach or suggest absolute quantitation of the catalytic subunit of telomerase mRNA that is specifically amplified from concentrated tumor cells from a body fluid as an indication of the quantity of tumor cells in a body fluid. Cech *et al.* also does not teach or suggest correlating the amount of the specifically amplified catalytic subunit of telomerase mRNA to the number of tumor cells in the sample. There is no teaching in Cech *et al.* of concentrating tumor cells that are present in a body fluid using a cell separation medium (*i.e.*, a suitable liquid of desired density as defined in the specification, *e.g.*, at page 18, lines 24-31). Further, Cech *et al.* does not teach or suggest any concentration of tumor cells by centrifugation after applying a cell separation medium to the body fluid. Furthermore, Cech *et al.* does not teach or suggest cell cultivation or separation steps to separate telomerase-positive nontumor cells from tumor cells.

Van Vlasselaer

Van Vlasselaer does not cure the deficiencies in the teachings of Cech. Van Vlasselaer is directed to a method for enriching breast tumor cells from body fluids. Van Vlasselaer describes a method for concentrating breast cancer cells using a "cell-trap" centrifugation tube and a density gradient solution such as Percoll or Ficoll in which the density of the solution is specifically adjusted to enrich for the breast tumor cells, **depending on the specific type of breast tumor cell**. Van Vlasselaer does not teach any diagnostic assay for the detection or quantification of tumor cells **in general** in a body fluid. Further, Van Vlasselaer does not teach or suggest amplification and quantitation of the mRNA for the catalytic subunit of telomerase, or any other mRNA, for the quantification of tumor cells in a body fluid. Furthermore, in the concentration method of Van Vlasselaer, the density of the medium is adjusted **according to breast cancer cell type**. Van Vlasselaer does not teach or suggest a concentration step as claimed in the instant methods, where concentration of the tumor cells is effected

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regardless of the tissue or cell type from which the circulating tumor cells originated, and there is no teaching or suggestion in Van Vlasselaer of the separation of tumor cells from telomerase-positive non tumor cells.

Hence, as discussed below, the combination of teachings of Cech *et al.* and Van Vlasselaer are defective in failing to teach or suggest (i) concentration of tumor cells from a body fluid by a method that is independent of tumor cell type; (ii) specific amplification of the catalytic subunit of telomerase from the tumor cells in a body fluid regardless of the tissue of origin of the tumor cells; (iii) the separation of tumor cells from telomerase-positive non tumor cells; and (iv) quantitation of the amplified catalytic subunit of telomerase for the quantification of tumor cells in a body fluid.

The combination of teachings of the cited references does not result in the instantly claimed methods.

The combination of teachings of the cited references does not result in the instantly claimed methods. None of the cited references, singly or in any combination thereof, teaches or suggests any method for quantifying circulating tumor cells in a body fluid (all pending claims). Neither teaches concentration of tumor cells from a body fluid regardless of tissue or tumor cell type, nor that the catalytic subunit of telomerase mRNA can be specifically amplified and used to quantitate tumor cells concentrated or from a body fluid. Neither reference teaches or suggests the concentration of tumor cells regardless of tumor cell type using a cell separation medium, nor the separation of tumor cells from telomerase-positive nontumor cells in body fluids such as blood (claims 71, 69, 70 and 83). Therefore, the combination of the cited references, each of which is missing critical elements of the instant claims, cannot result in the claimed methods.

Cech *et al.* teaches quantification of the gene encoding the catalytic subunit of telomerase as indicative of cancer when the level of expression in a biological sample is elevated relative to normal levels in that sample. Cech *et al.* does not teach or suggest enrichment of tumor cells in a body fluid; specifically

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amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase; and quantitating the specifically amplified mRNA to thereby quantitate the tumor cells in the body fluid. Van Vlasselaer, directed to a method for enriching breast tumor cells from body fluids, does not cure these deficiencies. Even if, as the Examiner alleges, Van Vlasselaer teaches the use of Percoll or Ficoll in centrifugation methods, there is no teaching or suggestion in Van Vlasselaer for the enrichment of tumor cells in general from a body fluid. The concentration step of the instantly claimed methods, which concentrates the tumor cells from a body fluid regardless of their tissue and cell type, and which, in the case of Claims 69 and 70 and added Claim 71, separates the tumor cells from the telomerase-positive non tumor cells, is not taught or suggested by the method in Cech *et al.*, which merely measures the catalytic subunit of telomerase in biological samples, nor by the method in Van Vlasselaer, which teaches adjusting the density of the gradient solution according to the type of breast cancer cell. Therefore, combinations of these references, both of which lack the elements of (i) concentrating tumor cells from a body fluid, regardless of tumor cell type; (ii) enriching for tumor cells regardless of tumor cell type by treatment with a cell separation medium; (iii) specifically amplifying mRNA for the catalytic subunit of telomerase from tumor cells in a body fluid, regardless of tumor tissue type and regardless of whether other cells in body fluid may express the same gene; and (iv) quantitating the specifically amplified mRNA to thereby quantitate tumor cells in a body fluid, cannot cure these deficiencies with respect to the instant claims.

Neither of the references nor their combination teaches or suggests that the catalytic subunit of telomerase can be specifically amplified and quantified in tumor cells, and that such quantitation can be used to detect and quantify tumor cells in body fluids as a diagnostic assay. Neither reference teaches or suggests assays of any sort for the quantification of tumor cells in general. Van Vlasselaer provides a method for the concentration of breast cancer cells from a body fluid, in which the density of the gradient is adjusted according to breast

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cancer cell type. Van Vlasselaer does not teach or suggest a general method for concentrating tumor cells in a body fluid, and Van Vlasselaer certainly does not use such concentration step in a method for the quantification of tumor cells in a body fluid by specific amplification of the catalytic subunit of telomerase.

Further, with respect to Claims 18, 19, 69 and 70, and added Claim 71, Van Vlasselaer does not teach or suggest selective cultivation or separation of tumor cells from telomerase-positive non tumor cells. Hence, Van Vlasselaer does not cure the principal deficiencies in the teachings of Cech *et al.*

The cited references, singly or in combination, fail to teach or suggest the missing elements of the claims. The combinations of teachings fail to suggest several elements of the claimed methods, including but not limited to, concentration of tumor cells from a body fluid regardless of tumor cell type by treatment with a cell separation medium and/or employing centrifugation after applying a separation medium to a body fluid, quantification of tumor cells in the body fluid by measuring the level of the catalytic subunit of telomerase specifically in the tumor cells, and selecting for or separating tumor cells from telomerase positive non-tumor cells.

Judicial Notice

Also, the Examiner cannot take judicial notice of facts outside the record that are not capable of instant and unquestionable demonstration. The Examiner makes quite a few such allegations. It is alleged that the following are obvious from what is "routinely practised in the art": (1) Percoll and Ficoll as cell separation media (limitations of instant Claim 23); (2) providing a substance that prevents platelets from sticking to the tumor cells and facilitates removal of the platelets (limitations of instant Claim 24); and (3) adjusting the density of the cell separation medium according to cell type, (limitations of instant claims 21, 22, 24 and 62-64).

It is noted that the Examiner cannot take official notice of facts outside the record that are not capable of instant and unquestionable demonstration.

The Examiner is reminded that MPEP 2144.03 states:

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The Examiner may take official notice of facts outside of the record which are capable of instant and unquestionable demonstration as being "well-known" in the art. *In re Ahlert*, 424 F.2d 1088, 1091, 165 USPQ 418, 420 (CCPA 1970). . . . (emphasis added).

MPEP 2144.03 goes on to provide, as examples of facts of which proper official notice can be taken, the "common practice to postheat a weld after the welding operation is completed, or "to adjust the intensity of a flame in accordance with the heat requirements" (*In re Ahlert*). These facts are in the nature of universal truths that are uniformly applicable and are guaranteed to work, irrespective of variations, in a given art.

To the contrary, the elements that the Examiner urges any one of ordinary skill in the art would have done in the context of the subject matter of this application are not "capable of instant and unquestionable demonstration" as being "well-known" in the art. All of the above "routine art" has been shown to be applicable to the instantly claimed method for the quantification of tumor cells in a body fluid only through Applicant's own efforts. None of the cited references teaches or suggests, in a method for quantification of tumor cells in a body fluid, (1) a correlation between the amount of mRNA for the catalytic subunit of telomerase and the quantity of tumor cells in a body fluid; (2) using a substance that prevents platelets from sticking to tumor cells before concentrating the tumor cells; or (3) using a cell separation medium such as Percoll or Ficoll for obtaining concentrated tumor cells from a body fluid where the density of the cell separation medium is adjusted to concentrate the tumor cells regardless of tumor cell type. Neither of the cited references teaches or suggests that the above "routine" practices would actually work in a method for the quantification of tumor cells in a body fluid. The "routine art" cited by the Examiner involves experimentation with various sets of conditions to optimize the steps of the claimed methods. For example, the selection of density of the cell separation medium to separate tumor cells regardless of the type of tumor cell is neither taught or suggested by the "routine" concept of choosing a density according to the characteristics of a particular type of cell. It is not

enough that adjusting medium density according to a cell type is known; the optimal densities for concentration of tumor cells from a body fluid regardless of cell type are only discovered through experimentation. Thus, the density of cell separation medium that produces a workable step in the claimed methods is neither "routine" nor "well-known". Further, the use of a substance that prevents platelets from sticking to tumor cells before concentrating the tumor cells does not automatically render the ability to detect a particular target gene, such as the catalytic subunit of human telomerase as claimed in the instant application, nor the quantification of tumor cells, obvious. The selection of a purification technique for biological samples is also neither "routine" nor "Well-known"; such selection is based on the nature of the sample, desired results and available equipment.

MPEP 2144.03 continues:

If justified, the examiner should not be obliged to spend time to produce documentary proof. If the knowledge is of such notorious character that official notice can be taken, it is sufficient so to state. *In re Malcolm*, 129 F.2d 529, 54 USPQ 235 (CCPA 1942). If the applicant traverses such an assertion the examiner should cite a reference in support of his or her position.

In this instance, a reference or references supporting assertions by the Examiner should be provided. But even if such reference(s) were provided, it would not establish obviousness because, as discussed above, the principal elements of the claimed subject matter, namely, a method for the quantification of tumor cells in a body fluid by (i) concentrating the tumor cells using a cell separation medium, regardless of tumor cell type, from the body fluid; (ii) in the cultivating or separating the tumor cells from the telomerase-positive non tumor cells; (iii) specifically amplifying, from the tumor cells and regardless of its tissue type, mRNA for the catalytic subunit of telomerase; and (iv) quantitating the amplified mRNA for the catalytic subunit of telomerase to thereby quantify the tumor cells in the body fluid; are lacking in the cited references.

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Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

B. REJECTION OF CLAIM 3 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF GWYNN *ET AL.*

Claim 3 is rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Gwynn *et al.* (U.S. Patent No. 6,025,156). It is alleged that it would have been *prima facie* obvious to one of skill in the art to have modified the method of generating cDNA for the catalytic subunit of telomerase allegedly taught by Cech *et al.* with using DNase for the removal of DNA from RNA samples as allegedly taught by Gwynn *et al.*, to arrive at the subject matter of Claim 3. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Relevant law

The relevant law is described above.

Analysis

Claim 3 is directed to the method of Claim 2, which in turn depends on Claim 1. Claim 3 specifies that in the method of Claim 1, *i.e.*, a method for the quantification of tumor cells in a body fluid as set forth above, the sample is treated with a DNase and then cDNA is prepared from the mRNA prior to amplification. Added Claims 71 and 72 are as set forth above.

Thus, Claim 3 is directed to a method for the quantification of tumor cells in a body fluid that recites the steps of (i) concentrating the tumor cells using a cell separation medium to obtain concentrated tumor cells; (ii) specifically amplifying, from the tumor cells, the mRNA for the catalytic subunit of telomerase; (iii) quantifying amplified cDNA prepared from a sample of mRNA for the catalytic subunit of telomerase that has been pre-treated with DNase; and (iv) correlating the quantity of amplified mRNA with the quantity of tumor cells in a body fluid. Added Claim 71 recites that the tumor cells are concentrated and separated from telomerase positive non-tumor cells; and added Claim 72

recites that the tumor cells are concentrated in the first step by treating the sample of body fluid with a cell separation medium.

Teachings of the cited art and differences between the teachings of the cited art and Claim 3

Cech et al.

The teachings of *Cech et al.* are discussed above.

Gwynn et al.

Gwynn et al. is directed to topoisomerase III polypeptides, DNA and RNA encoding the polypeptides and recombinant techniques for producing such polypeptides. *Gwynn et al.* teaches diagnostic assays for detecting the topoisomerase III polypeptides and diseases related to the presence of the polypeptides in a host. *Gwynn et al.* also teaches methods for using topoisomerase III and/or its antagonists for treating infections. *Gwynn et al.* teaches that prior to reverse transcription of RNA to obtain cDNA encoding topoisomerase III, the RNA sample is treated with DNase to remove traces of DNA.

The teachings of *Gwynn et al.* are unrelated to the instant claims, including Claim 3. *Gwynn et al.* does not teach or suggest any method for the quantitation of tumor cells, much less quantitation by measuring expression of the catalytic subunit of telomerase. *Gwynn et al.* also does not teach or suggest any enrichment or depletion of tumor cells, and there is no teaching or suggestion of using a cell separation medium. While *Gwynn et al.* may teach removal of DNA by DNase treatment prior to reverse transcription of RNA, there is no teaching or suggestion of reverse transcribing and specifically amplifying, from tumor cells, mRNA encoding any gene, including the catalytic subunit of telomerase.

The combination of teachings of the cited references does not result in the instantly claimed methods

The combination of teachings of the cited references does not result in the instantly claimed methods. As discussed above, Cech *et al.* does not teach or suggest a method for quantifying circulating tumor cells in a body fluid that are concentrated from a body fluid by treatment with a cell separation medium. Further, Cech *et al.* does not teach or suggest any concentration of tumor cells by centrifugation after applying a cell separation medium to the body fluid. Cech *et al.* does not teach or suggest specifically amplifying, from the tumor cells, mRNA encoding the catalytic subunit of telomerase, nor quantitation of the amplified mRNA to thereby quantitate the number of tumor cells in a body fluid. Gwynn *et al.*, directed to Topoisomerase III, does not cure these deficiencies. Even if, as the Examiner alleges, Gwynn *et al.* teaches treatment of mRNA samples with DNase prior to reverse transcription to obtain cDNA, Gwynn *et al.* does not provide any teaching for the detection and quantification of tumor cells, much less any of the steps of the methods as instantly claimed. Therefore, a combination of the cited references, both of which lack the elements of (i) concentrating tumor cells from a body fluid by treatment with a cell separation medium and/or employing centrifugation after applying a separation medium to a body fluid; and (ii) specifically amplifying mRNA for the catalytic subunit of telomerase from the tumor cells in a body fluid and quantitating the amplified mRNA to thereby quantitate the tumor cells, cannot cure these deficiencies with respect to the instant claims. Neither of the references nor their combination teaches or suggests that the catalytic subunit of telomerase may be specifically amplified and quantified in tumor (as opposed to non-tumor) cells, and that such quantitation may be used to detect and quantify tumor cells in body fluids as a diagnostic assay. Further, neither of the references nor their combination teaches or suggests any method that includes a step of separating tumor cells from telomerase positive non-tumor cells.

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Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

C. REJECTION OF CLAIM 29 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF SELBY

Claim 29 is rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Selby (Great Britain Patent No. GB 2 260 811). It is alleged that it would have been *prima facie* obvious to one of skill in the art to combine the method of Cech *et al.* with Selby, which allegedly teaches diagnosis or monitoring of cancer by the detection of mRNA in a sample such as blood where the cancer cells are not normally present, to arrive at the subject matter of Claim 29. It is also alleged that Selby teaches cooling after centrifugation, which is an element of Claim 29, was routinely practiced in the art. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Relevant law

The relevant law is described above.

Analysis

Claim 29 is dependent on Claim 1, which includes the step of concentration of tumor cells that is effected by (i) covering a cell separation medium with a layer of the body fluid; (ii) centrifuging the cell separation medium covered with the body fluid; and (iii) collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid. Claim 29 specifies that in the method of Claim 1, after centrifugation and before collecting the tumor cell-enriched interface, the centrifugation vessel is removed and cooled to prevent mixing of the cells in the different layers. Added claims 71 and 72 are as set forth above.

Thus, Claim 29 is directed to a method for the quantification of tumor cells in a body fluid that includes the steps of (i) covering a cell separation medium with a layer of the body fluid; (ii) centrifuging the cell separation

medium covered with the body fluid; (iii) cooling the centrifugation vessel; (iv) collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid; (v) concentrating the tumor cells using a cell separation medium and collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid; (vi) specifically amplifying, from the tumor cells, the mRNA for the catalytic subunit of telomerase; and (vii) quantifying the amplified mRNA for the catalytic subunit of telomerase and correlating the quantity of amplified mRNA with the quantity of tumor cells in a body fluid.

Added Claim 71 recites that the tumor cells are concentrated and separated from telomerase positive non-tumor cells; and added Claim 72 recites that the tumor cells are concentrated in the first step by treating the sample of body fluid with a cell separation medium.

Teachings of the cited art and differences between the teachings of the cited art and Claim 29

Cech *et al.*

The teachings of Cech *et al.* are discussed above

Selby

Selby is directed to methods for diagnosing malignant tumors in which **total** mRNA is extracted from a sample of body fluid and reverse transcribed into cDNA, which is amplified using primers based upon a **tissue-specific gene** not normally expressed in the body fluid, followed by analysis to determine whether such amplified cDNA is present. Selby does not suggest using a gene for amplification that is not tissue specific nor does Selby suggest quantification of tumor cells in a body fluid. Selby thus teaches using a tissue-specific gene, not a gene that is ubiquitously expressed.

Further, Selby does not teach a method for concentrating or depleting tumor cells from a body fluid; Selby merely teaches the separation of plasma from all blood cells for isolating RNA from the blood cells (which may include tumor cells from a specific tissue that are then detected in a tissue-specific

manner, as discussed above). Not only does Selby not teach or suggest the concentration of tumor cells from a body fluid, Selby does not teach or suggest any method for detecting all tumor cells in a body fluid, regardless of their cell type, much less that the level of of the catalytic subunit of telomerase, specifically amplified from concentrated tumor cells, is indicative of the presence of tumor cells in general in the body fluid.

Selby is directed to a method for detecting a **tissue-specific mRNA** in a sample using RT-PCR, and is of no relevance to the instantly claimed methods. Selby does not suggest anything about the catalytic subunit of telomerase nor suggest that it can be used for quantification of tumor cells in a sample of body fluid. Selby does not teach or suggest that the amount of the catalytic subunit of telomerase can be specifically amplified and quantitated in tumor cells concentrated from a body fluid, nor that such amount is related to the number of tumor cells in the fluid.

The combination of teachings of the cited references does not result in the instantly claimed methods

The combination of teachings of the cited references does not result in the instantly claimed methods. As discussed above, Cech *et al.* does not teach or suggest a method for quantifying circulating tumor cells in a body fluid that are concentrated from a body fluid by treatment with a cell separation medium and/or by employing centrifugation steps after applying a cell separation medium to a body fluid. Cech *et al.* does not teach or suggest specifically amplifying, from the tumor cells, mRNA encoding the catalytic subunit of telomerase, nor quantitation of the amplified mRNA to thereby quantitate the number of tumor cells in a body fluid. Selby, directed to diagnosis or monitoring of cancer using coamplification to detect and quantify tissue-specific genes, does not cure these deficiencies.

Even if, as the Examiner alleges, Selby teaches cooling samples after centrifugation, none of its teachings cure the deficiencies in the teachings of the Cech *et al.* so that their combination fails to provide a method for the

quantification of tumor cells in a body fluid. Neither of the cited references, singly or in combination, provide any teaching or suggestion of a method for the quantification of tumor cells in a body fluid in which (i) the tumor cells are concentrated from the body fluid, regardless of cell type; (ii) the mRNA for the catalytic subunit of telomerase is specifically amplified from the tumor cells, regardless of their tissue of origin; and (iii) the amount of amplified mRNA of the catalytic subunit of telomerase can be correlated with the quantity of tumor cells in the body fluid. Further, the concentration step and its elements set forth in Claim 1, from which Claim 29 depends, and added Claim 72 and claims dependent thereon, use a cell separation medium to concentrate tumor cells from a body fluid regardless of their tissue and cell type. The treatment of a body fluid with a cell separation medium and/or employing centrifugation steps after applying a cell separation medium to a body fluid is not taught or suggested by the method in Selby, which merely separates plasma from blood, nor by Cech *et al.*, which merely teaches measurement of the catalytic subunit of telomerase in a biological sample and the normalization of such measurement to the total number of cells in the sample.

Therefore, combinations of these references, both of which lack the elements of (i) concentrating tumor cells from a body fluid, regardless of tumor cell type by treatment and/or centrifugation with a cell separation medium; and (ii) specifically amplifying mRNA for the catalytic subunit of telomerase from tumor cells in a body fluid, regardless of tumor tissue type and regardless of whether other cells in body fluid may express the same gene, cannot cure the deficiencies with respect to Claim 29, nor with respect to added Claim 72 and claims dependent thereon. With respect to added Claim 71 and claims dependent thereon, neither of the cited references, singly or in combination, teaches or suggests a step of separating tumor cells from telomerase positive non-tumor cells.

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Judicial Notice

Also, the Examiner cannot take judicial notice of facts outside the record that are not capable of instant and unquestionable demonstration. It is alleged that Selby teaches that cooling after centrifugation was "routinely practiced in the art."

As discussed above, MPEP 2144.03 states:

The Examiner may take official notice of facts outside of the record which are capable of instant and unquestionable demonstration as being "well-known" in the art. *In re Ahlert*, 424 F.2d 1088, 1091, 165 USPQ 418, 420 (CCPA 1970). . . .

The practice of cooling the samples after centrifugation has been shown to be applicable to the instantly claimed method for the quantification of tumor cells in a body fluid only through Applicant's own efforts. None of the cited references teaches or suggests, in a method for quantification of tumor cells in a body fluid, cooling centrifuged body fluid samples to separate layers of tumor cells from non tumor cells after centrifugation. Therefore, as discussed above, a reference or references supporting applicability of this step to the instantly claimed method should be provided. But even if such reference(s) were provided, it would not establish obviousness because, as also discussed above, the principal elements of the claimed subject matter, namely, a method for the quantification of tumor cells in a body fluid by (i) concentrating the tumor cells, regardless of tumor cell type, from the body fluid; (ii) specifically amplifying, from the tumor cells and regardless of its tissue type, mRNA for the catalytic subunit of telomerase; and (iii) quantitating the amplified mRNA for the catalytic subunit of telomerase to thereby quantify the tumor cells in the body fluid; are lacking in all the cited references.

Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

**D. REJECTION OF CLAIMS 5 AND 6 UNDER 35 U.S.C. § 103(a)
OVER CECH *ET AL.* IN VIEW OF GELMINI *ET AL.***

Claims 5 and 6 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Gelmini *et al.* (*Clin. Chem.*, 43(5): 752-758 (1997)). It is alleged that it would have been *prima facie* obvious to one of skill in the art to combine the method of Cech *et al.* with the amplification method of Gelmini *et al.*, which allegedly teaches real time quantitative PCR. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Relevant law

The relevant law is described above.

Analysis

The Claims

Dependent Claim 5 specifies that in the method of Claim 1 for the quantification of tumor cells in a body fluid, the amplification products of the catalytic subunit of telomerase are labeled during amplification and the amplification kinetics are measured continuously, including during the amplification process. Claim 6 further specifies that a probe that is specific for the amplification products and that emits a characteristic signal proportional to the products amplified per synthesis cycle, is present during amplification. Added Claims 71 and 72 are as set forth above.

Thus, Claims 5 and 6 are directed to a method for the quantification of tumor cells in a body fluid that includes the steps of (i) concentrating the tumor cells using a cell separation medium; (ii) specifically amplifying, from the tumor cells, the mRNA for the catalytic subunit of telomerase; (iii) quantifying the amplified mRNA for the catalytic subunit of telomerase by monitoring amplification product-specific probes and/or labeled amplification products; and (iv) correlating the quantity of amplified mRNA with the quantity of tumor cells in a body fluid. Added Claim 71 recites that the tumor cells are concentrated

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and separated from telomerase positive non-tumor cells; and added Claim 72 recites that the tumor cells are concentrated in the first step by treating the sample of body fluid with a cell separation medium.

None of the references teaches or suggests a method for the quantification of tumor cells in a body fluid, much less by treatment and/or centrifugation with a cell separation medium. Therefore, a combination of the cited references cannot cure this deficiency. Further, none of the cited references, singly or in combination, lead to the combination of steps (i) - (iii) of the instantly claimed methods. Furthermore, none of the cited references, singly or in any combination, teaches or suggests separation of tumor cells from telomerase-positive non tumor cells.

As discussed below, not only do none of the cited references, singly or in any combination, teach or suggest a method for the quantification of tumor cells in a body fluid by specifically amplifying the mRNA for the catalytic subunit of telomerase, there is also no teaching or suggestion in any of the references or combinations thereof for the separation of tumor cells in a body fluid from telomerase-positive non tumor cells.

Teachings of the cited art and differences between the teachings of the cited art and Claims 5 and 6

Cech *et al.*

The teachings of Cech *et al.* are discussed above

Gelmini *et al.*

Gelmini *et al.* is directed to polymerase chain reaction (PCR)-based homogeneous assays in which fluorogenic probes containing a reporter dye and a quencher dye are used to quantitate amplification of the *c-erbB-2* oncogene from human breast tumors. Gelmini *et al.* teaches that during the extension phase of the PCR cycle, the 5'--> 3' exonuclease activity of *Taq* polymerase cleaves the hybridized fluorogenic probes resulting in an increase of fluorescence emission of the reporter dye. Gelmini *et al.* further teaches that the increased

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fluorescence emission of the reporter dye is quantitative for the amount of PCR product.

Gelmini *et al.* does not teach or suggest any method for the quantitation of tumor cells, much less quantitation by measuring expression of the catalytic subunit of telomerase. Gelmini *et al.* only teaches measurement of the *c-erbB-2* oncogene in a specific type of tumor cell, namely, breast cancer cells. Gelmini *et al.* does not teach or suggest quantification of any gene, such as the catalytic subunit of telomerase, that is amplified from tumor cells regardless of the tumor cell type. Gelmini *et al.* also does not teach or suggest any enrichment or concentration of tumor cells, and there is no teaching or suggestion of using a cell separation medium. While Gelmini *et al.* may teach real-time quantitative PCR in the context of quantitation of the *c-erbB-2* oncogene in breast cancer cells, there is no teaching or suggestion of a method for quantification of tumor cells in a body fluid by specifically amplifying, from concentrated tumor cells, mRNA encoding any gene, including the catalytic subunit of telomerase. Gelmini *et al.* also does not teach or suggest any method that includes a step of separating tumor cells from telomerase positive non-tumor cells.

The combination of teachings of the cited references does not result in the instantly claimed methods

The combination of teachings of the cited references does not result in the instantly claimed methods. As discussed above, Cech *et al.* does not teach or suggest a method for quantifying circulating tumor cells in a body fluid that are concentrated from a body fluid by treatment and/or centrifugation with a cell separation medium. Cech *et al.* does not teach or suggest specifically amplifying, from the tumor cells, mRNA encoding the catalytic subunit of telomerase, nor quantitation of the amplified mRNA to thereby quantitate the number of tumor cells in a body fluid. Gelmini *et al.*, directed to quantitative PCR of the *c-erbB-2* oncogene in breast cancer cells, does not cure these deficiencies. Even if, as the Examiner alleges, Gelmini *et al.* teaches quantitative PCR using labelled amplification products, Gelmini *et al.* does not

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provide any teaching for the detection and quantification of tumor cells, much less any of the steps of the methods of Claims 5 and 6. Gelmini *et al.* also does not teach or suggest treatment with a cell separation medium as set forth in Claims 5, 6 and 72, and Gelmini *et al.* certainly does not teach or suggest any method that includes a step of separating tumor cells from telomerase positive non-tumor cells. Therefore, a combination of the cited references, both of which lack the elements of (i) concentrating tumor cells from a body fluid by treatment and/or centrifugation with a cell separation medium; and (ii) specifically amplifying mRNA for the catalytic subunit of telomerase from the tumor cells in a body fluid and quantitating the amplified mRNA to thereby quantitate the tumor cells, cannot cure these deficiencies with respect to Claims 5 and 6 and added Claim 72. Neither of the references nor their combination teaches or suggests that the catalytic subunit of telomerase may be specifically amplified and quantified in tumor (as opposed to non-tumor) cells, and that such quantitation may be used to detect and quantify tumor cells in body fluids as a diagnostic assay. With respect to added Claim 71, neither of the cited references, singly or in combination, teaches or suggests any method that includes a step of separating tumor cells from telomerase positive non-tumor cells.

Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

**E. REJECTION OF CLAIM 13 UNDER 35 U.S.C. § 103(a) OVER
CECH *ET AL.***

Claim 13 is rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* It is alleged that it would have been *prima facie* obvious to one of skill in the art to select primers to amplify all or part of the catalytic subunit of telomerase gene using the parameters according to "routine methods" allegedly taught by Cech *et al.* Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

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Relevant law

The relevant law is described above.

Analysis

Claim 13 specifies that in the method of Claim 1 for the quantification of tumor cells in a body fluid, the amplification primers whose sequences are set forth in SEQ ID. NOS. 1 and 2 are used for the amplification of the catalytic subunit of telomerase mRNA. Added claims 71 and 72 are set forth above.

Thus, Claim 13 is directed to a method for the quantification of tumor cells in a body fluid that includes the steps of (i) concentrating the tumor cells using a cell separation medium; (ii) specifically amplifying, from the tumor cells, the mRNA for the catalytic subunit of telomerase using primers whose sequences are set forth in SEQ. ID. NOS. 1 and 2; (iii) quantifying the amplified mRNA for the catalytic subunit of telomerase; and (iv) correlating the quantity of amplified mRNA with the quantity of tumor cells in a body fluid. Added Claim 71 recites that the tumor cells are concentrated and separated from telomerase positive non-tumor cells; and added Claim 72 recites that the tumor cells are concentrated in the first step by treating the sample of body fluid with a cell separation medium.

Teachings of the cited art and differences between the teachings of the cited art and Claim 13

Cech *et al.*

The teachings of Cech *et al.* are discussed above.

The combination of the teachings of Cech *et al.* with those of the "routine art" does not result in the instantly claimed methods

It is alleged that the primers whose sequences are set forth in SEQ ID. NOS. 1 and 2 and that are elements of Claim 13 were merely selected by the "routine methods" provided by Cech *et al.* for the amplification of all or part of the catalytic subunit of telomerase. As discussed above, however, Cech *et al.* does not teach or suggest principal elements of Claim 13 or of added Claim 72, such as a method for quantifying circulating tumor cells in a body fluid that are

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concentrated from a body fluid by treatment and/or centrifugation with a cell separation medium. Cech *et al.* does not teach or suggest specifically amplifying, from the tumor cells, mRNA encoding the catalytic subunit of telomerase, nor quantitation of the amplified mRNA to thereby quantitate the number of tumor cells in a body fluid.

At col. 106, lines 45-68, Cech *et al.* teaches that primers are "typically between about 12 and about 50 bases, and often between about 14 and about 25 bases in length." Cech *et al.* further provides, in Table 2, illustrative primers for PCR amplification of the catalytic subunit of telomerase. Cech *et al.* provides a "laundry list" of primer lengths and suggested sequences for amplifying the catalytic subunit of telomerase. Cech *et al.* provides no teaching or suggestion as to selection of particular sequences of primers that may be used in a method for the quantification of tumor cells by specific amplification of the catalytic subunit of telomerase. There is no teaching or suggestion in Cech *et al.* that the selection of primers with sequences set forth in SEQ ID NOS. 1 and 2 would be particularly desirable for quantitating tumor cells by specific amplification of the catalytic subunit of telomerase. In fact, Cech *et al.* teaches away from using primers with sequences set forth in SEQ ID NOS. 1 and 2 because none of the suggested primer sequences set forth in Table 2 of Cech *et al.* correspond to the primer sequences that are elements of Claim 13.

Even if, as the Examiner alleges, Cech *et al.* provides primers for the quantitative amplification of the catalytic subunit of telomerase and parameters for their selection, Cech *et al.* does not provide any teaching for the detection and quantification of tumor cells, much less any of the steps of the methods of Claim 13. With respect to added Claim 71, Cech *et al.* does not provide any teaching or suggestion of a method that includes a step of separating tumor cells from telomerase positive non-tumor cells.

Judicial Notice

Also, as discussed above, the Examiner cannot take judicial notice of facts outside the record that are not capable of instant and unquestionable

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demonstration. It is alleged that Cech *et al.* teaches "routine methods" for the selection of primers to amplify all or a part of the catalytic subunit of telomerase gene.

The Examiner is reminded that MPEP 2144.03 states:

The Examiner may take official notice of facts outside of the record which are capable of instant and unquestionable demonstration as being "well-known" in the art. *In re Ahlert*, 424 F.2d 1088, 1091, 165 USPQ 418, 420 (CCPA 1970). . . .

As also discussed above, MPEP 2144.03 goes on to provide, as examples of facts of which proper official notice can be taken, the "common practice to postheat a weld after the welding operation is completed, or "to adjust the intensity of a flame in accordance with the heat requirements" (*In re Ahlert*). These facts are in the nature of universal truths that are uniformly applicable and are guaranteed to work, irrespective of variations, in a given art.

To the contrary, the "routine art" that the Examiner urges any one of ordinary skill in the art would have done in the context of the subject matter of this application are not "capable of instant and unquestionable demonstration" as being "well-known" in the art. The "routine art" cited by the Examiner involves experimentation with various sets of conditions to optimize the steps of the claimed methods. For example, the success of the primers chosen for amplification of a gene in the context of a method, such as one involving concentration and quantification of tumor cells in a body fluid, depends largely on the particular primers selected, which is subject to experimental trial and error. Cech *et al.* provides no teaching or suggestion as to selection of particular primers for an assay for the quantification of tumor cells in a body fluid. It is not enough that the sequence of the target nucleic acid to be amplified or of suggested lists of primer sequences and their preferred lengths be known; the optimal primers sites are only discovered through experimentation with various primer sequences. Moreover, as discussed above, Cech *et al.* does not even provide the primer sequences that are elements of Claim 13 as part of its list of suggested sequences from which they could be

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"selected". Thus, the construction of primers that produce a workable step in the claimed subject matter is neither "routine" nor "well-known. The above "routine art" has been shown to be applicable to the instantly claimed method for the quantification of tumor cells in a body fluid only through Applicant's own efforts.

MPEP 2144.03 continues:

If justified, the examiner should not be obliged to spend time to produce documentary proof. If the knowledge is of such notorious character that official notice can be taken, it is sufficient so to state. In re Malcolm, 129 F.2d 529, 54 USPO 235 (CCPA 1942). If the applicant traverses such an assertion the examiner should cite a reference in support of his or her position.

In this instance, a reference or references supporting assertions by the Examiner should be provided. But even if such reference(s) were provided, it would not establish obviousness because, as discussed above, the principal elements of Claim 13, namely, a method for the quantification of tumor cells in a body fluid by (i) concentrating the tumor cells, regardless of tumor cell type, from the body fluid using a cell separation medium; (ii) specifically amplifying, from the tumor cells, mRNA for the catalytic subunit of telomerase; and (iii) quantitating the amplified mRNA for the catalytic subunit of telomerase to thereby quantify the tumor cells in the body fluid; are lacking in Cech *et al.*

Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

F. REJECTION OF CLAIMS 12 AND 57-59 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF MELVIN *ET AL.*

Claims 12 and 57-59 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Melvin *et al.* (WO 97/12246). It is alleged that it would have been *prima facie* obvious to one of skill in the art to have modified the method of Cech *et al.* to include controls such as β -actin as a positive control and sterile water as a negative control as allegedly taught by Melvin *et al.*, to arrive at the claimed subject

matter. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Relevant law

The relevant law is described above.

Analysis

The Claims

Claim 12 is directed to the method of Claim 1 in which water is employed as a negative control. Claim 57 is directed to the method of Claim 11, which specifies that the sample used in the method of Claim 1 is peripheral blood. Claim 57 specifies that as a positive control, a nucleic acid that occurs in peripheral blood is specifically amplified and detected. Claim 58 further specifies positive controls such as β -globin, glyceraldehyde phosphate dehydrogenase, β -actin or a T-cell receptor. Claim 59 is directed to the method of Claim 3 where no reverse transcription reaction is carried out before amplification and/or water is used in the amplification as a negative control. Added claims 71 and 72 are set forth above.

Thus, the claims are directed to a method for the quantification of tumor cells in a body fluid that includes the steps of (i) concentrating the tumor cells by treatment and/or centrifugation with a cell separation medium; (ii) specifically amplifying, from the tumor cells, the mRNA or the cDNA for the catalytic subunit of telomerase using appropriate positive and negative controls; (iii) quantifying the amplified mRNA for the catalytic subunit of telomerase; and (iv) correlating the quantity of amplified mRNA with the quantity of tumor cells in a body fluid; in which the positive and negative controls used in the amplification reactions are specified. Added Claim 71 includes a step of separating tumor cells from telomerase positive non-tumor cells.

Teachings of the cited art and differences between the teachings of the cited art and Claims 12 and 57-59

Cech et al.

The teachings of *Cech et al.* are discussed above

Melvin et al.

Melvin et al. is directed to the identification of a cytochrome P450 form, CYP1B1, in a wide range of human cancers. *Melvin et al.* provides a diagnostic method that includes the steps of: (a) obtaining from a patient a tissue sample to be tested for the presence of cancer cells; (b) producing a prepared sample in a sample preparation process; (c) contacting the prepared sample with an antibody that reacts with human CYP1B1 protein; and (d) detecting binding of the antibody to CYP1B1 protein in the prepared sample. In RT-PCR experiments to detect CYP1B1 mRNA, *Melvin et al.* teaches that β -actin may be used as a positive control and sterile water may be used as a negative control (page 17).

Melvin et al. does not teach or suggest any method for the quantitation of tumor cells in a body fluid, much less quantitation by measuring specific expression of the catalytic subunit of telomerase in tumor cells that are concentrated from the body fluid by treatment and/or centrifugation with a cell separation medium. *Melvin et al.* only teaches measurement of CYP1B1 in cancer cells. While *Melvin et al.* may teach use of sterile water and β -actin as negative and positive controls respectively in RT-PCR experiments, there is no teaching or suggestion of a method for quantification of tumor cells in a body fluid by specifically amplifying, from concentrated tumor cells, mRNA encoding the catalytic subunit of telomerase. *Melvin et al.* also does not teach or suggest any method that includes a step of separating tumor cells from telomerase positive non-tumor cells.

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The combination of teachings of the cited references does not result in the instantly claimed methods

The combination of teachings of the cited references does not result in the instantly claimed methods. As discussed above, Cech *et al.* does not teach or suggest a method for quantifying circulating tumor cells in a body fluid that are concentrated from a body fluid by treatment and/or centrifugation with a cell separation medium. Cech *et al.* does not teach or suggest specifically amplifying, from the tumor cells, mRNA encoding the catalytic subunit of telomerase, nor quantitation of the amplified mRNA to thereby quantitate the number of tumor cells in a body fluid. Cech *et al.* also does not teach or suggest any method that includes a step of separation of tumor cells from telomerase positive non-tumor cells. Melvin *et al.*, directed to the detection of CYP1B1 in cancer cells, does not cure these deficiencies. Even if, as the Examiner alleges, Melvin *et al.* teaches the use of β -actin and sterile water as positive and negative controls respectively in RT-PCR experiments, Melvin *et al.* does not provide any teaching for the detection and quantification of tumor cells, much less any of the steps of the methods of Claims 12 and 57-59. Therefore, a combination of the cited references, both of which lack the elements of (i) concentrating tumor cells from a body fluid by treatment and/or centrifugation with a cell separation medium; and (ii) specifically amplifying mRNA for the catalytic subunit of telomerase from the tumor cells in a body fluid and quantitating the amplified mRNA to thereby quantitate the tumor cells, cannot cure these deficiencies with respect to Claims 12 and 57-59 and added Claim 72. Neither of the references nor their combination teaches or suggests that the catalytic subunit of telomerase may be specifically amplified and quantified in tumor (as opposed to non-tumor) cells, and that such quantitation may be used to detect and quantify tumor cells in body fluids as a diagnostic assay. With respect to added Claim 71, neither of the references, singly or in combination, teaches or suggests any method that includes the step of separating tumor cells from telomerase positive non-tumor cells.

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Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

G. REJECTION OF CLAIMS 30-33, 65 AND 66 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF VAN VLASSELAER AND OKA *ET AL.*

Claims 30-33, 65 and 66 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Van Vlasselaer and further in view of Oka *et al.* (U.S. Patent No. 5,298,165). It is alleged that it would have been *prima facie* obvious to one of skill in the art to have modified the method of Cech *et al.* for the quantitation of tumor cells in view of the enrichment of tumor cells allegedly taught by Van Vlasselaer, with different membranes, filters or porous barriers allegedly taught by Oka *et al.*, to arrive at the subject matter of the rejected claims. It is further alleged that the pore size and thickness of filters are "routinely optimizable" based upon the desired parameters, since Oka *et al.* allegedly teaches how densities may be determined. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Relevant law

The relevant law is described above.

Analysis

The Claims

Claim 30 is directed to the method of Claim 1 in which the centrifugation vessel is divided into two compartments by a porous barrier, filter or sieve, and the body fluid is introduced into the upper compartment. Claims 31, 32, 65 and 66 specify the pore size of the porous barrier, filter or sieve, and Claim 33 specifies that at least one of the porous barrier, filter or sieve is fabricated from or coated with a hydrophobic material. Added Claims 71 and 72 are set forth above.

Thus, the claims are directed to a method for the quantification of tumor cells in a body fluid that includes the steps of (i) concentrating the tumor cells

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by centrifugation after applying a cell separation medium to a body fluid in a centrifugation vessel that is divided into two compartments by a porous barrier, filter or sieve; (ii) specifically amplifying, from the tumor cells, the mRNA or the cDNA for the catalytic subunit of telomerase using appropriate positive and negative controls; (iii) quantifying the amplified mRNA for the catalytic subunit of telomerase; and (iv) correlating the quantity of amplified mRNA with the quantity of tumor cells in a body fluid. Added Claim 71 recites that the tumor cells are concentrated and separated from telomerase positive non-tumor cells; and added Claim 72 recites that the tumor cells are concentrated in the first step by treating the sample of body fluid with a cell separation medium.

Teachings of the cited art and differences between the teachings of the cited art and Claims 30-33, 65 and 66

Cech *et al.*; Van Vlasselaer

The teachings of Cech *et al.* and Van Vlasselaer are discussed above

Oka *et al.*

Oka *et al.* is directed to a filter system and method for removing leukocytes from a leukocyte-containing blood product. Oka *et al.* teaches that removal of leukocytes from a blood product prior to transfusion reduces the side effects of transfusion such as headache, chills, fever and nausea. The method provided by Oka *et al.* includes steps of passing the leukocyte-containing blood product through microfilters having a non-woven or woven fabric containing fibers with an average diameter of 0.3 to 1.6 μm .

The teachings of Oka *et al.* are unrelated to the instant claims. Oka *et al.* does not teach or suggest any method for the quantitation of tumor cells in a body fluid, much less quantitation by measuring specific expression of the catalytic subunit of telomerase in tumor cells that are concentrated from the body fluid by treatment and/or centrifugation with a cell separation medium. Oka *et al.* only teaches removal of leukocytes from leukocyte-containing blood products. While Oka *et al.* may use filters in its methods, there is no teaching or suggestion of a method for quantification of tumor cells in a body fluid by

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specifically amplifying, from concentrated tumor cells, mRNA encoding the catalytic subunit of telomerase. Oka *et al.* also does not teach or suggest any method that includes a step of separating tumor cells from telomerase positive non-tumor cells.

The combination of teachings of the cited references does not result in the instantly claimed methods

As discussed above, neither Cech *et al.* nor Van Vlasselaer, singly or in combination, teaches or suggests a method for the quantification of tumor cells in a body fluid where the tumor cells are concentrated from the body fluid regardless of tumor tissue type by treatment and/or centrifugation with a cell separation medium. Neither of the references, singly or in combination, teaches or suggests that the specifically amplified catalytic subunit of telomerase can be correlated with the quantity of tumor cells in a body fluid. Further, neither of the references, singly or in combination, teaches or suggests concentrating tumor cells regardless of tumor cell type using a centrifugation vessel that is divided into two compartments by a porous barrier, filter or sieve. Neither Cech *et al.* nor Van Vlasselaer, singly or in combination, teaches or suggests that the catalytic subunit of telomerase expressed by tumor cells in a body fluid may be specifically amplified in the presence of non tumor cells in a body fluid, nor whether non tumor cells in a body fluid express the catalytic subunit of telomerase. Further, with respect to added Claim 71, neither Cech *et al.* nor Van Vlasselaer, singly or in combination, teaches or suggests any method that includes a step of separating tumor cells from telomerase positive non-tumor cells.

Oka *et al.*, directed to removal of leukocytes from leukocyte-containing blood products to reduce the side effects of blood transfusions, fails to cure these deficiencies. Oka *et al.* does not teach or suggest any assay for the detection of tumor cells in a body fluid, nor does Oka *et al.* teach or suggest quantification of tumor cells. While Oka *et al.* may provide for the use of filters in its methods, Oka *et al.* does not teach or suggest concentration of tumor cells

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in a body fluid, much less by treatment and/or centrifugation with a cell separation medium. Further, Oka *et al.* does not teach or suggest any method that includes a step of separating tumor cells from telomerase positive non tumor cells. Hence, Oka *et al.* does not cure the principal deficiencies in the teachings of Cech *et al.* and Van Vlasselaer.

Hence the combinations of teachings fail to suggest several elements of the claimed methods, including but not limited to, concentration of tumor cells from a body fluid regardless of tumor cell type by treatment and/or centrifugation with a cell separation medium, quantification of tumor cells in the body fluid by measuring the level of the catalytic subunit of telomerase specifically in the tumor cells, and separation of tumor cells from telomerase positive non-tumor cells.

Judicial Notice

Also, as discussed above, the Examiner cannot take judicial notice of facts outside the record that are not capable of instant and unquestionable demonstration. It is alleged that Oka *et al.* teaches that pore size and thickness of filters are "routinely optimizable" based upon the desired parameters.

As discussed above, MPEP 2144.03 states:

The Examiner may take official notice of facts outside of the record which are capable of instant and unquestionable demonstration as being "well-known" in the art. *In re Ahlert*, 424 F.2d 1088, 1091, 165 USPQ 418, 420 (CCPA 1970). . . .

The selection of barriers, filters and sieves of suitable pore sizes has been optimized to the instantly claimed method for the quantification of tumor cells in a body fluid only through Applicant's own efforts. None of the cited references teaches or suggests, in a method for quantification of tumor cells in a body fluid, selection of suitable filters that are elements of the instant claims. The "routine art" cited by the Examiner, namely, selection of pore sizes and filters, involves experimentation with various sets of conditions to optimize the steps of the claimed methods. The selection of an optimum purification technique for enriching or depleting tumor cells from a body fluid so that the

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catalytic subunit of telomerase may be specifically amplified from tumor cells and not from telomerase positive non-tumor cells is neither "routinely optimizable" nor "well-known"; such selection is based on the nature of the sample, desired results and available equipment.


Accordingly, as discussed above, a reference or references supporting applicability of this step to the instantly claimed method should be provided. But even if such reference(s) were provided, it would not establish obviousness because, as also discussed above, the principal elements of the claimed subject matter, namely, a method for the quantification of tumor cells in a body fluid by (i) concentrating the tumor cells, regardless of tumor cell type, from the body fluid; (ii) specifically amplifying, from the tumor cells and regardless of its tissue type, mRNA for the catalytic subunit of telomerase; and (iii) quantitating the amplified mRNA for the catalytic subunit of telomerase to thereby quantify the tumor cells in the body fluid; are lacking in all the cited references.

Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

* * *

In view of the remarks herein, reconsideration of the requirement for restriction and examination of all claims on the merits are respectfully requested.

Respectfully submitted,
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Dahm *et al.*

Serial No.: 09/601,645

Confirmation No.: 7793

Filed: August 4, 2000

For: *METHOD FOR THE QUANTITATIVE
DETERMINATION OF TUMOR CELLS IN
A BODY FLUID AND TEST KITS
SUITABLE THEREOF*

Art Unit: 1634

Examiner: Goldberg, J.A.

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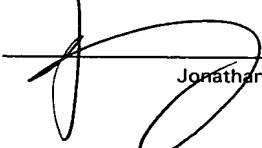
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Jonathan Ong

ATTACHMENTS TO THE AMENDMENT

1. Marked up copy of specification and claims pursuant to 37 C.F.R. §1.121.



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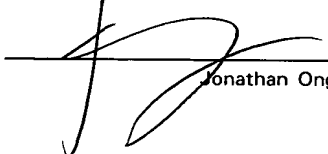
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Jonathan Ong

MARKED UP CLAIMS (37 C.F.R. § 1.121)

Please amend claims 1, 21-24, 26-30, 34, 61, 62, 64, 69 and 70 as follows:

1. (Amended three times) A method for the quantification of tumor cells in a body fluid, comprising:

(a) concentrating [or depleting] tumor cells in a sample of a body fluid by covering a cell separation medium with a layer of the body fluid, centrifuging the cell separation medium covered with the body fluid and collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid;

(b) specifically amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase; and

(c) quantitatively determining the amount of amplified nucleic acid, thereby quantifying tumor cells in a body fluid.

21. (Amended twice) The method of Claim [20] 1, wherein the cell separation medium has a density in the range of from 1.060-1.067 g/ml.

22. (Amended twice) The method of Claim [20] 1, wherein the centrifugation is carried out at about 1000 x g for about 30 minutes.

23. (Amended twice) The method of Claim [20] 1, wherein the cell separation medium used is Percoll or Ficoll.

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24. (Amended twice) The method of Claim [20] 1, wherein the body fluid is blood and prior to applying the body fluid sample to the cell separation medium, the body fluid is mixed with one or more substances that prevent aggregation of platelets to tumor cells, and/or prior to applying the body fluid sample to the cell separation medium, the body fluid is freed of substances that promote aggregation of platelets to tumor cells.

26. (Amended twice) The method of Claim [25] 11, wherein the peripheral blood is drawn in an anticoagulant substance and, prior to covering the cell separation medium, diluted with a diluent.

27. (Amended twice) The method of Claim [25] 11, wherein the peripheral blood is venous or arterial blood.

28. (Amended twice) The method of [Claim 20] 1, wherein the body fluid is selected from the group consisting of lymph, urine, exudates, transudates, spinal fluid, seminal fluid, saliva, fluids from natural or unnatural body cavities, bone marrow and dispersed body tissue.

29. (Amended three times) The method of Claim [20] 1, wherein after centrifugation and before collecting the tumor-cell-enriched interface, the centrifugation vessel is removed and cooled to prevent mixing of the cells in the different layers.

30. (Amended twice) The method of Claim [20] 1, wherein the centrifugation is carried out in a vessel that is divided by a porous barrier, a filter or a sieve into an upper and a lower compartment and the body fluid is introduced into the upper compartment.

34. (Amended three times) The method of Claim [20] 1, wherein a dye is added to color the cell separation medium, whereby the color of the cell separation medium is distinguishable from that of the supernatant body fluid.

61. (Amended) The method of Claim [17] 78, wherein concentration is effected by immunoabsorption.

62. (Amended) The method of Claim [20] 1, wherein the cell separation medium has a density in the range of from 1.055 to < 1.070 g/ml.

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64. (Amended) The method of Claim [25] 11, wherein the peripheral blood is drawn in an anticoagulant substance and, prior to covering the cell separation medium, diluted with a diluent at a ratio of about 1:1.

69. (Amended) The method of Claim [20] 1, wherein the tumor cells are separated from telomerase-positive non tumor cells.

70. (Amended) The method of Claim [20] 1, further comprising, before step (i), adjusting the density of the cell separation medium and thereby separating tumor cells from telomerase-positive non tumor cells.